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IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :

NOBUYA MATSUOKA, ET AL. : EXAMINER: AMY LEWIS

SERIAL NO: 10/716,865 :

FILED: NOVEMBER 20, 2003 : GROUP ART UNIT: 1614

for: METHOD FOR THE TREATMENT OF PARKINSON'S DISEASE COMPRISING ADMINISTERING AN A_1A_{2a} RECEPTOR DUAL ANTAGONIST :

DECLARATION UNDER 37 C.F.R. § 1.132

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

Now comes NOBUYA MATSUOKA who deposes and states that:

1. I am a graduate of Kyoto University, Faculty of Pharmaceutical Sciences and received my Ph.D. degree in the year 1992.

2. I have been employed by Fujisawa Pharmaceutical Co. (now Astellas Pharma) for 23 years as a pharmacologist in the field of neuroscience.

3. That I understand the English language or, at least, that the contents of the Declaration were made clear to me prior to executing the same.

4. The following experiments were carried out by me or under my direct supervision and control.

5. The data shown in the Table 1 below were obtained by the following methods.

Test 1: Adenosine receptor binding assay (columns 3- 5)

The adenosine antagonistic activity [Ki(nM)] of the test compound was examined by radioligand binding techniques. Membrane fractions prepared from the CHO cells which transfected stably with human recombinant A_1 or A_{2a} receptors were incubated for 1 hr at 25°C

with test compounds, ADA, 50 mM Tris buffer and [^{3}H]-DPCPX (final 4 nM) or [^{3}H]-CGS21680 (final 15 nM), respectively. Reaction mixtures were filtrated with 96-well harvestor to separate free ligands from bound fraction using GF/C filter. Radioactivity of the dried filter was counted, and specific binding of each labeled ligands was calculated.

Test 2 : Anticataleptic action in mice (column 6)

The test compound was administered orally with ddY mice. Then, haloperidol (0.32mg/kg) was injected intraperitoneally 30 mm, after the administration of the compound. Thirty min. after the injection, the cataleptic responses of mice were measured. The forelimbs of each mouse were placed on a 3 cm high, 3 mm wide horizontal bar, and the duration of cataleptic posture was measured for up to 30 sec.

Test 3: Social interaction test in rats (column 7)

Two male SD rats acclimated for 1 week and handled once were used in sets. One hour before beginning of the test, both rats in each set were orally dosed with the same amount of the test compound or the vehicle. Immediately thereafter, both rats were placed in a new environment where territories were to be established as yet and their behaviors were monitored and recorded over 15 minutes. After the test, the records were analyzed and evaluated in terms of the total duration of social interaction (social interaction time) (observation items: sniffing, following, grooming, kicking, boxing, biting, wrestling, crawling under or over the partner).

Test 4: Elevated plus maze test in rats (column 8)

Elevated plus-maze test apparatus (NS-M506; Neuroscience Co., Tokyo, Japan) consisted of a plus-shaped gray-colored maze elevated 50 cm from the floor. It comprised two open arms (50 x 10 cm) and two enclosed arms (50 x 10 x 45 cm), arranged so that the arms of the same type were opposite each other, and connected by an open central area (platform; 10 x

10 cm). A video camera for monitoring rats was suspended above the center of the maze. Behavioral testing was conducted in a darkened sound-proof room and the maze was lit obliquely from above, giving 10 lux at the ends of the open arms. Testing was conducted during the light part of the light-dark cycle. Testing started by placing the rat onto the central platform facing an open arm 1 hr after oral administration of vehicle or agents. The observation started immediately thereafter and lasted 5 min. The number of entries of the whole animal (four paws) and the time spent in each arm were recorded from a video monitor in real time. The total number of entries (open + closed), the percentage of open arm entries (100 x open / total) were calculated. The time spent in open arms, the percentage of open arm entries and the number of open arm entries were regarded as indices of anxiolytic effect.

Test 5 Impaired memory ameliorating action (column 9)

Male Wistar rats were serially subjected to a habituation trial, an acquisition trial 1 hour after the habituation trial, and a retention trial 24 hours after the acquisition trial. Scopolamine 1 mg/kg was intraperitoneally administered 30-minutes before the acquisition trial. The test compound was intraperitoneally administered immediately after the acquisition trial.

In brief, a two-compartment step-through passive avoidance apparatus made of black perspex was used. The apparatus consisted of illuminated and dark compartments attached to a grid floor and were separated by a guillotine door. The rat was placed in the illuminated compartment and the door was raised. After entering the dark compartment, the rat was returned to its home cage (habituation trial). In the scopolamine-treated model, rats were given an i.p. injection of scopolamine (1 mg/kg) 30 mm after the habituation trial, and after further 30 minutes, the rat was again placed in the illuminated compartment (acquisition trial). When the rat entered the dark compartment, the guillotine door was closed. Scrambled electrical foot shocks with intensity of 0.4 mA was delivered for 4 sec through the grid floor using a shock generator (Neuroscience Co., Tokyo, Japan; model NS-SGO1). Compound was administered

i.p. immediately after the acquisition trial (60 mm after the habituation trial). In the test trial made 24 h after the acquisition trial, the rat was placed again in the illuminated compartment and the response lately to enter the dark compartment was measured up to a maximum of 300 sec (retention trial). The results were recorded as average latency for each group of rats. The percentage of rats reaching criterion (300 sec) was also calculated.

Test 6: Spontaneous locomotor activity measurement in rats (column 10)

Locomotor activity was assessed in white acrylic cage (35 cm x 29 cm x 18 cm in height). The test cages were placed on each external sensor unit (ANIMEX). Oscillator circuits in the external sensors generated an electrical field; the sensitivity of the meter was adjusted so that it recorded the number of times the field was disrupted only by gross movements, such as locomotion in any direction and rearing. On the test day, the rats were weighed and immediately placed in the test cages for a 60mm habituation period after oral administration of test drugs.

*; The data represent the statistically significant effective dose in each experiment.

Table 1. Comparison of efficacies of adenosine antagonists

Adenosine antagonist	Compound	Adenosine receptor binding assay (Ki:nM)			Anticataleptic action (ED50:mg/kg)	Anxiolytic action (Effective dose:mg/kg)		Impaired-memory ameliorating action (Effective dose:mg/kg)	Locomotor activity* (Effective dose:mg/kg)
		A_1	A_{2a}	A_{2a}/A_1		Rat social interaction test*	Rat elevated plus-maze*	Scopolamine-rat passive avoidance test*	
A_1 selective	A	6.6	5400	818	≥10	3.2	10	0.32, 1.0	No increase
A_{2a} selective	B	≥287	9.12	<0.03	0.078	1, 3.2, 10	0.32, 1, 3.2, 10	No effect	0.32, 3.2, 10, 32
Dual	C	0.51	15.6	30.4	0.36	0.32	1, 3.2	0.1, 0.32, 1, 3.2	No increase
	D	0.35	2.46	7.03	0.40	3.2	---	---	3.2, 10
	E	0.11	0.80	7.27	0.11	0.32,1	1, 3.2, 10	0.1, 0.32, 1, 3.2	0.1, 0.32, 1, 3.2
	F	0.36	1.39	3.86	0.06	---	1, 3.2	0.1, 0.32, 1	---
	G	0.43	0.42	0.98	0.075	0.1, 0.32, 1	0.1, 0.32, 1.	0.1	0.032, 0.1, 0.32, 1, 3.2, 10
	H	16.15	3.95	0.26	0.276	32	3.2, 10, 32	1, 3.2, 10	---

---; not tested,

6. The undersigned petitioner declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

7. Further deponent saith not.

Nobuya Matsuoka
Signature

Dec. 11, 2006
Date

Customer Number
22850
Tel. (703) 413-3000
Fax. (703) 413-2220
(OSMMN 05/06)

TMC/law